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Investigation of protein phosphorylation and protein kinases in prokaryotes

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Methylococcus capsulatus (Bath) is a Gram-negative, coccoid, methanotrophic bacterium. For the utilization of methane *M. capsulatus* is able to express two methane monooxigenases (MMO): in the presence of copper ions the particulate MMO (pMMO) and its accessory and transport proteins, responsible for copper uptake, are expressed. In the absence of copper the soluble MMO (sMMO) is expressed. sMMO can oxidize a wide range of compounds, from alkanes, alkenes, ethers and haloalkanes to aromatic and even heterocyclic hydrocarbons (Hakemian et al. 2007). Many biodegradation and biotransformation applications for sMMO are currently being investigated.

Although the existence of protein phosphorylation on S, T and Y residues in prokaryotes was first demonstrated in 1978 (Wang et al. 1978), our knowledge about S, T and Y phosphorylation in prokaryotes is very limited. In this recent work the copper regulation of MMO enzymes is studied by comparing the phospoproteome of two cultures grown under distinct conditions and screening for proteins of which's phosphorylation state changes depending on the available copper.

The comparison of the purified phosphoproteomes on 2D ELFO revealed that two subunits of sMMO (smmoB and smmoC) are phosphorylated proteins and unstable elongation factor (EfTU) is only phosphorylated when the media contains no copper. In case of smmoB and EfTU exact phosphorylation sites (smmoB:ser2, EfTU ser144) were determined by mass spectrometry. After changing potential phosphorylation site on smmoB from ser2 to ala by directed mutagenesis the whole enzyme preserved its full activity and smmoB still remained phosphorylated. Furthermore even smmoB heterologously expressed in *E. coli* proved to be phosphorylated by host protein kinases. In order to identify the protein kines(es) that is(are) responsible for the phosphorylation of smmoB a set of kinase deletion mutants were prepared in *E. coli*. After deletion of all known ser/thr and tyr kinases (*yihE, argK, aceK, etk, wzc, hipA, yeaG, yniA*) *E. coli* still preserved its capability to perform protein phosphorylation and smmoB was still phosphorylated, furthermore the deletion of these kinases hardly affected the protein pattern of the whole phosphoproteome of the host bacterium. Although *E. coli* is one of the most studied organisms, its genome is known and well characterized these results suggest that it still may possesses at least one unknown functional protein kinase that is responsible for the phophorylation of the majority of phosphpoproteins including overxpressed smmoB.

During amino acid starvation bacteria activate stringent control elements that result in adaptation to the amino acid shortage by increased amino acid synthesis, restricted protein translation and intensive protein degradation (Chatterji et al. 2001). In *Methylococcus capsulatus* activation of stringent control cascade results in the activation of smmo operon even in copper rich media (unpublished results). Promoting amino acid starvation in *M. capsulatus* grown in copper rich medium also resulted the phosphorylation of EfTU suggesting that phosphorylation of this protein may restrict protein sinthesys *via* direct or indirect inhibition of the translation.

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A novel genetic approach for Identifying genes involved in abscisic acid regulation

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Abscisic acid is the main stress response hormone in higher plants. In the past few decades many stress regulatory factors were identified which are involved in ABA dependent stress regulation. In order to understand the complicated regulatory web of ABA signaling the Controlled cDNA Overexpression System have been developed (COS, Papdi et al., 2008). We have transformed the *Arabidopsis* Col-0 wild type plants with the COS library and screened progenies of infiltrated plants for ABA insensitivity in the presence and absence of estradiol in germination assays. Screening one million seeds (aproximately 25,000 transformed seeds), of T1 generation resulted 156 plants, which were selected based on their germination capacity on high concentration ABA supplemented media. By testing of T2 generation, estradiol dependent ABA insensitivity was confirmed in 32 lines. Estradiol dependent ABA insenstitive germination was most notable in A26 and A44 lines, which were able to germinate in the presence of 5μM ABA, which otherwise completely inhibited the germination of wild type seeds. Insertions were identified in both lines and corresponded to full-length cDNA encoding the small heat-shock protein HSP17.6A-cII (A26) and a previously unknown zinc-finger domain containing transcription factor protein (A44). GFP fusion and HA-tagging experiments showed nuclear localization of the A44-derived transcription factor. While constitutive overexpression of this transcription factor reduced